SECURITY CLASSIFICATION OF THIS PAGE

REPORT DOCUMENTATION PAGE

1a. REPORT SECURITY CLASSIFICATION UNCLASSIFIED	1b. RESTRICTIVE MARKINGS
2a. SECURITY CLASSIFICATION AUTHORITY	3. DISTRIBUTION/AVAILABILITY OF REPORT Approved for public release; distribution unlimited
2b. DECLASSIFICATION/DOWNGRADING SCHEDULE	
4. PERFORMING ORGANIZATION REPORT NUMBER(S)	5. MONITORING ORGANIZATION REPORT NUMBER(S)
6a. NAME OF PERFORMING ORGANIZATION University of Virginia 6b. OFFICE SYMBOL (If applicable)	7a. NAME OF MONITORING ORGANIZATION
6c. ADDRESS (City, State, and ZIP Code) School of Medicine Charlottesville, Virginia 22908	7b. ADDRESS (City, State, and ZIP Code)
8a. NAME OF FUNDING/SPONSORING ORGANIZATION U.S. Army Medical Research & Development Command 8b. OFFICE SYMBOL (If applicable)	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER DAMD17-83-G-9565
8c. ADDRESS (City, State, and ZIP Code) Fort Detrick Frederick, Maryland 21701-5012	10. SOURCE OF FUNDING NUMBERS PROGRAM ELEMENT NO. 61102A; PROJECT NO. 3M1-61102BS12; TASK NO. AA; WORK UNIT ACCESSION NO. 110

11. TITLE (Include Security Classification)
STUDIES OF THE BIOLOGICAL AND MOLECULAR BASIS OF THE INHIBITION OF ACTIVITY OF PHAGOCYTIC CELLS BY ANTHRAX TOXIN

12. PERSONAL AUTHOR(S)
Wright, G.G., Mandell, G.L., Hewlett, E.L.

13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
Annual	FROM 83/9/30 TO 84/9/29	1985 March	29

16. SUPPLEMENTARY NOTATION

17. COSATI CODES			18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
FIELD	GROUP	SUB-GROUP	
06	13		Anthrax Toxin, Chemotaxis, Polymorphonuclear Neutrophils, Respiratory burst, Adenylate Cyclase
06	03		

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

This report presents investigations on the effects of the toxin of Bacillus anthracis on human polymorphonuclear neutrophils. The toxin stimulated chemotaxis and inhibited oxidative activity. The toxin components protective antigen plus edema factor, but not protective antigen plus lethal factor, produced small increases in 3'5'-adenosine monophosphate (cyclic AMP).

20. DISTRIBUTION/AVAILABILITY OF ABSTRACT ☑ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT. ☐ DTIC USERS	21. ABSTRACT SECURITY CLASSIFICATION UNCLASSIFIED
22a. NAME OF RESPONSIBLE INDIVIDUAL Jane B. Iodine	22b. TELEPHONE (Include Area Code) 301/663-7325
	22c. OFFICE SYMBOL SGRD-RMS

DD FORM 1473, 84 MAR

83 APR edition may be used until exhausted.
All other editions are obsolete.

SECURITY CLASSIFICATION OF THIS PAGE

(12)

AD_____

STUDIES OF THE BIOLOGICAL AND MOLECULAR BASIS OF THE INHIBITION OF ACTIVITY OF PHAGOCYTIC CELLS BY ANTHRAX TOXIN

Annual Report

George G. Wright, Gerald L. Mandell, and Erik L. Hewlett

March 1985

Supported by
U. S. Army Medical Research and Development Command
Fort Detrick, Frederick, Maryland 21701-5012

Grant No. DAMD17-83-G-9565

Division of Infectious Diseases
Department of Internal Medicine
The University of Virginia
Charlottesville, Va. 22908

AD_____

STUDIES OF THE BIOLOGICAL AND MOLECULAR BASIS OF THE INHIBITION OF ACTIVITY OF PHAGOCYTIC CELLS BY ANTHRAX TOXIN

Annual Report

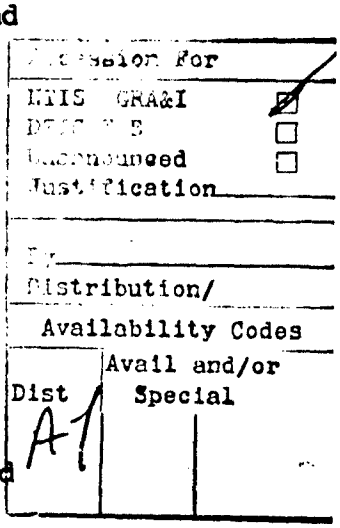
George G. Wright, Gerald L. Mandell, and Erik L. Hewlett

March 1985

Supported by
U. S. Army Medical Research and Development Command
Fort Detrick, Frederick, Maryland 21701-5012

Grant No. DAMD17-83-G-9565

Division of Infectious Diseases
Department of Internal Medicine
The University of Virginia
Charlottesville, Va. 22908



Summary

This report presents investigations of the effects of the toxin of Bacillus anthracis on phagocytic cells, and the biochemical reactions associated with these effects. Combinations of protective antigen (PA) plus edema factor (EF), and PA plus lethal factor (LF) markedly stimulated chemotaxis of human polymorphonuclear neutrophils (PMN), but had little effect on random migration. These effects were accompanied by a marked inhibition of the oxidative activity of these cells, as measured by a decrease in the formation of chloramines. PA + EF, but not PA + LF, produced a small but consistent increase in 3'5'- adenosine monophosphate (cAMP) in PMN; the levels of cAMP were markedly lower than those produced by PA + EF in certain tissue culture cells, and those produced by the adenylate cyclase of Bordetella pertussis in PMN. Keywords: Respiratory burst.

Foreword

Citations of commercial organizations and trade names in this report do not constitute an official Department of the Army endorsement or approval of the products or services of these organizations.

For the protection of human subjects the investigator(s) have adhered to policies of applicable Federal Law 45CFR46.

Table of Contents

Section I: Anthrax Toxin Components Stimulate Chemotaxis of Human Polymorphonuclear Neutrophils

Introduction. Anthrax toxin consists of three protein components: protective antigen (PA), edema factor (EF), and lethal factor (LF), each with a molecular weight of approximately 80,000 (1). PA is the major antigen involved in immunity to anthrax, and is the active component in non-viable anthrax vaccine (2). Intravenous administration of LF + PA produces death in many species of animals (3). EF + PA produces edema when injected into the skin. The complete toxin has antiphagocytic and antibactericidal effects, and enhances the virulence of attenuated strains of B. anthracis (4). Recently, it has been found that EF is an adenylate cyclase, which is activated by a heat-stable eucaryotic protein, calmodulin (5). EF + PA produces high levels of adenosine 3'-5'monophosphate (cAMP) in Chinese Hamster Ovary (CHO) cells (5).

Chemotaxis, the directed migration of cells along a chemical gradient, has been studied extensively with polymorphonuclear neutrophils (PMN) and shown to be a receptor-mediated process that involves assembly and organization of microtubules and localization of microfilaments (6). Biochemical events that follow receptor binding include release of bound calcium, influx of external calcium, and activation of complex pathways involving production of arachidonate and its metabolism to produce hydroxy-eicosatetraenoic acids, prostaglandins, and thromboxanes. Chemotaxis is depressed by cholera toxin (7), Escherichia coli enterotoxin (8), and urea extracts of Bordetella pertussis (9), all agents that promote formation of cAMP in phagocyte cells. Because of the adenylate cyclase activity of EF + PA, we hypothesized that the anthrax toxin also would inhibit chemotaxis of PMN, an effect that could contribute to the antiphagocytic activity observed by Keppie et al (4). We observed instead a marked enhancement of chemotaxis.

Materials and Methods.

Toxin. The three components were prepared as described previously (1), and resembled previous lots in biological and chemical properties. Stock solutions of the toxin components were stored at -70°C. B. pertussis urea extracts were prepared by the method of Confer and Eaton (9).

Chemotaxis. The agarose method of Nelson et al. (10) was used, with minor modifications described previously (8), except that PMN migration was measured from a magnified projection of the plates on the focusing screen of a microscope camera adaptor. Results are expressed as actual migration distance in millimeters, toward a well containing chemoattractant in the case of directed migration, toward an opposite well containing control buffer in the case of random migration. Agarose for Electrophoresis, manufactured by Litex, Denmark, was used. Human peripheral blood neutrophils (PMN) were obtained by gravity sedimentation of heparinized venous blood. The PMN were incubated for 60 minutes at 37°C in a shaker waterbath with toxin components. The chemoattractant formyl-methionyl phenylalanine (FMP) (Sigma Chemical Co.) was used at a concentration of 10^{-4}M. It was dissolved in dimethylsulfoxide and diluted 1:1000 in aqueous buffer; this avoided any effect of dimethylsulfoxide on chemotaxis. Limited experiments in which 10^{-7}M formyl-methionyl-leucyl-phenylalanine was used as chemoattractant produced similar effects (data not shown). Zymosan-activated serum was prepared as described by Ward et al. (11).

cAMP Production. PMN were obtained from normal humans by the gravity sedimentation procedure used in chemotaxis studies, or by Ficoll-Hypaque separation, dextran sedimentation, and hypotonic lysis of erythrocytes (12). PMN at a final concentration of 5×10^6/ml were incubated for 1 hour at 37°C with toxin components at 1μg/ml, separately and in various combinations. Cells were sedimented by centrifugation and extracted with 0.1N

hydrochloric acid. The extracts were acetylated and cAMP determined by radioimmunoassay (13). Results are expressed as p moles cAMP per $5x10^6$ PMN. Similar results were obtained with the two types of cell preparation, and combined data are presented.

Results.

Six experiments using PMN from four donors are summarized in Fig. 1. Open bars represent directed migration and hatched bars represent random migration. None of the components alone caused a significant change in either random or directed migration. In contrast, the combinations of EF + PA, LF + PA, and LF + EF + PA each caused significant increases in directed migration toward FMP. The presence of PA appeared to be an absolute requirement since cells treated with EF alone, LF alone, or the combination of EF and LF were not different from controls. The effects of EF + PA and LF + PA were not reversed by two washes of the toxin-treated PMN with Hanks' balanced salt solution prior to measurement of chemotaxis. There was no significant change in random migration with any of the components or combinations.

To determine whether the enhancement of chemotaxis was specific for chemoattractant peptides, experiments were performed using Zymosan-activated serum; results were similar to those obtained using FMP.

The effects on random and directed migrations of different concentrations of PA, EF + PA, LF + PA, and EF + LF + PA were explored to determine whether the stimulation of chemotaxis noted at 1 μg per ml was evident over a broad range of concentrations (Fig. 2). Stimulation of chemotaxis by the combinations evidently reached a maximum in the range 0.3 to 1 μg per ml. Further increase produced no change in the case of LF + PA, but appeared to decrease the response to EF + PA and EF + LF + PA. The changes in random migration, and the effects of PA alone were minor and were not significant in the more extensive experiments summarized in Fig. 1.

To determine whether the toxin components were themselves

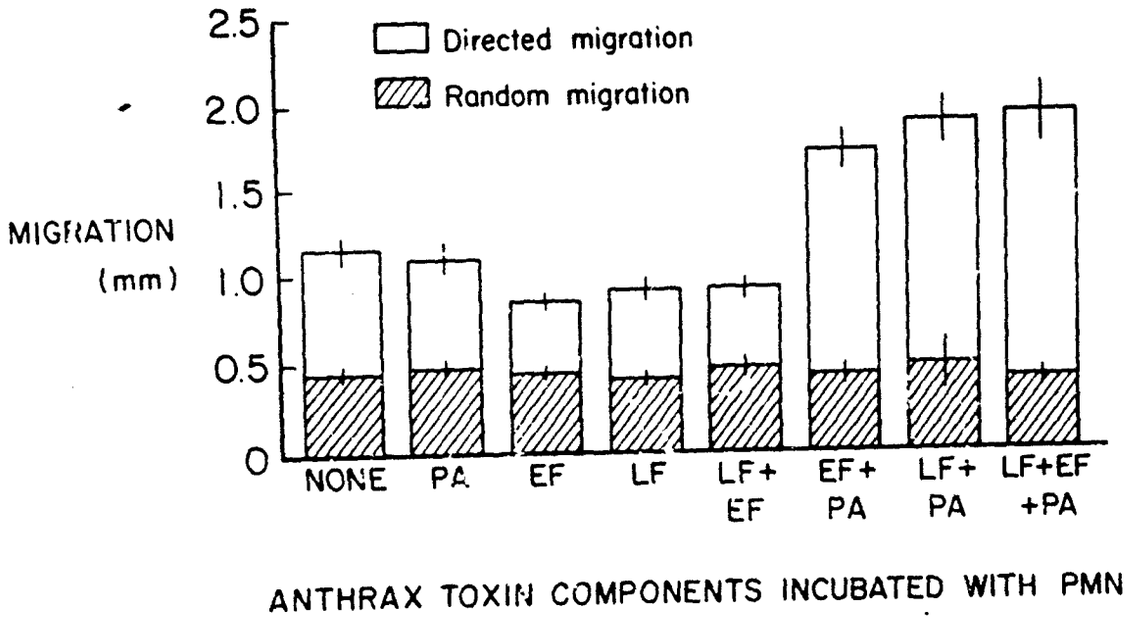


Fig. 1. Directed migration (open bars) and random migration (hatched bars) of human neutrophils after treatment with anthrax toxin components. Neutrophils, $5x10^6$, suspended in 1.0 ml of Hanks' balanced salt solution containing 1 µg/ml each of one or more anthrax toxin components were incubated for 60 minutes in a 37°C waterbath shaker, then concentrated 5-fold by centrifugation and allowed to migrate under agarose. Results are expressed as mm of migration from the edge of the well. The anthrax toxin components in each PMN preparation are shown beneath each bar. Directed migration was significantly enhanced by LF plus PA, EF plus PA, and LF plus EF plus PA ($p < 0.001$).

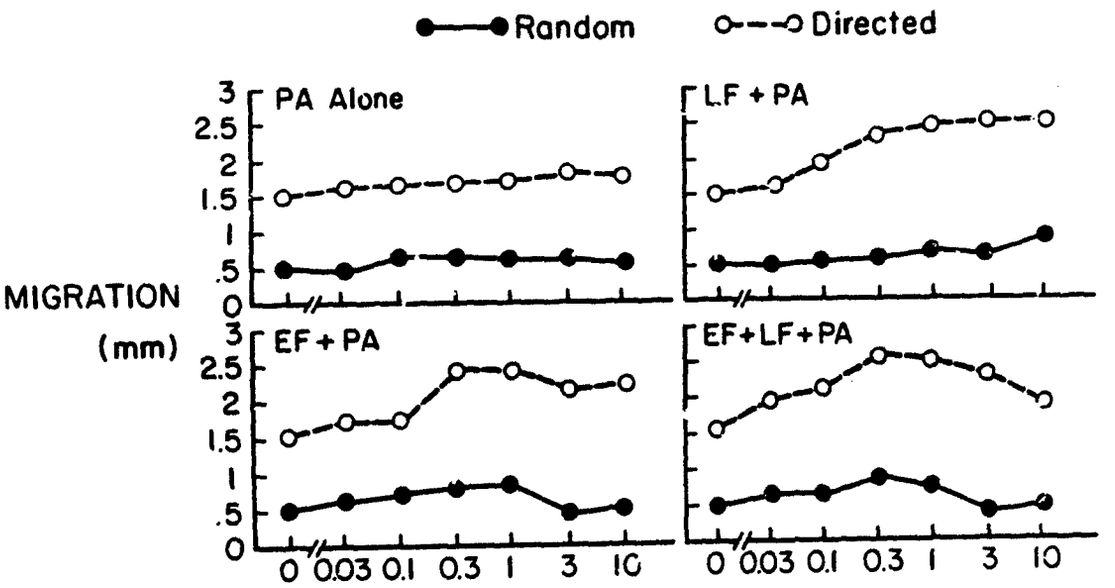


Fig. 2. Directed migration (upper curves) and random migration (lower curves) of human polymorphonuclear neutrophils incubated with a range of concentrations of anthrax toxin components indicated in the figure. The ordinates are least square means of migration distances for 3 to 6 wells. Otherwise, experimental conditions were the same as for Fig. 1.

chemoattractants, PMN were put in the center well and their migration toward the outer well containing each of the anthrax toxin components alone or in combination was measured. Migration toward the toxin components did not exceed random migration.

Measurements of cAMP in PMN treated with toxin components, alone and in various combinations, are summarized in Table I. A concentration of 1 µg/ml was used for each component, since this had produced maximum effects on chemotaxis. B. pertussis urea extract was included as a positive control.

Only those combinations of components containing EF + PA produced appreciable elevation of cAMP. All cAMP elevations produced by anthrax toxin were <1% of those produced by urea extract from B. pertussis.

TABLE I

Effect of Anthrax Toxin and Bordetella pertussis Urea Extract on cAMP Levels in Human PMN

Substance Added to PMN[a]	pmol cAMP per $5x10^6$ PMN
Control	3.41 ± 0.27
PA	2.90 ± 0.14
EF	4.37 ± 0.59
LF	2.50 ± 0.065
EF+PA	17.40 ± 1.26
LF+PA	5.48 ± 0.78
EF+LF+PA	10.10 ± 4.13
B. pertussis urea extract	>2000.00

[a] concentration of anthrax toxin components was 1 µg/ml. Dilution of B. pertussis urea extract was 1:10.

Discussion. The observed stimulation of chemotaxis (directed migration) but not of unstimulated random migration represents a new biological activity of the anthrax toxin which could provide clues to the nature of its antiphagocytic effect and its contribution to virulence of B. anthracis. The fact that the three toxin components were purified by repeated chromatographic procedures and approached homogeneity does not in itself eliminate the possibility that impurities were responsible for the observed effect. However, the absence of effects on chemotaxis by the individual toxin components makes this unlikely.

Early observations on the nature of this toxin tended to emphasize the requirement for cooperative action of the three components to produce lethal and antiphagocytic effects (4, 14), although PA + EF was evidently sufficient for production of cutaneous edema. More recent evidence has supported separate activities of EF and LF, which may be shown to compete for the sites of cell uptake formed by combination of PA with cell receptors (1). The present evidence that both EF + PA and LF + PA stimulate chemotaxis again raises the possibility that the three components act in a cooperative, or even a synergistic manner under appropriate conditions.

Exposure of PMN to EF + PA did not yield the high concentrations of cAMP observed after similar treatment of Chinese Hamster Ovary cells (5), nor the levels produced in PMN by B. pertussis urea extract as reported previously (9) and confirmed in the present work. The relatively low levels of cAMP produced in PMN were obtained at concentrations of EF + PA that produced maximum stimulation of chemotaxis. These low levels are consistent with the absence of inhibition of chemotaxis, but their relationship to the observed stimulation is not yet clear. The high levels of cAMP produced by pertussis

extract abolished chemotaxis (9).

A possible basis for the observed stimulation of chemotaxis by the toxin is provided by observations that relate secretory products of PMN to modulation of chemotaxis (15, 16). The toxin may inhibit exocytosis or respiratory burst activity, interfere with the modulation process, and allow unrestrained chemotactic migration. Whatever the mechanism and pathophysiological significance of the observed stimulation of chemotaxis, anthrax toxin would appear to represent a useful probe for study of leukocyte chemotaxis.

Summary. Effects of the three-component toxin of Bacillus anthracis on chemotaxis of human polymorphonuclear leukocytes (PMN) were investigated in an effort to determine the basis of the reported antiphagocytic effect of the toxin. The three toxin components, edema factor (EF), protective antigen (PA), and lethal factor (LF), were tested alone and in various combinations for their effect on PMN chemotaxis under agarose to formyl peptides and zymosan-activated serum. No component was active alone; combinations of EF + PA, LF + PA, and EF + LF + PA, markedly stimulated chemotaxis (directed migration), but had little or no effect on unstimulated random migration. The toxin components were not themselves chemoattractants. EF in combination with PA had previously been identified as an adenylate cyclase in Chinese Hamster Ovary (CHO) cells. We found that EF + PA produced detectable cyclic adenosine 3'-5'monophosphate (cAMP) in PMN, but the level of cAMP was less than 1% of that produced in CHO cells by EF + PA, and in PMN by other bacterial adenylate cyclases. LF + PA (which stimulated chemotaxis to an equivalent extent) had no effect on cAMP levels. Thus, the enhancement of chemotaxis by anthrax toxin (at least by LF + PA) does not seem to be related to adenylate cyclase activity.

Section II. Inhibition by Toxin of the Oxidative Activity of Human Neutrophils

In the preceeding section it was proposed that the stimulation of chemotaxis observed after treatment of human polymorphonuclear neutrophils (PMN) with PA + EF or PA + LF was a result of interference with the mechanisms that normally modulate the chemotactic response. These modulative mechanisms involve the secretory events that follow exposure of PMN to a variety of soluble or particulate stimuli, and include release to the extracellular fluid of substances capable of inactivating chemotactic substances, and of modifying

the surface properties and accordingly the chemotactic and other responses of PMN themselves. (15, 16)

Among the most important and extensively studied of the released substances are the components of the myeloperoxidase system, consisting of myeloperoxidase, hydrogen peroxide (H_2O_2), and an oxidizable cofactor such as halide ion. The H_2O_2 is produced by action of superoxide dismutase on superoxide anion (O_2^-), which is the initial product of the respiratory burst. (17). Myeloperoxide then catalyzes oxidation of halide ion by H_2O_2; chloride is oxidized to hypochlorite which reacts rapidly with oxidizable substances in the complex system. Evidently, endogenous amines, especially taurine, are the most active of the available receptors, and are converted to the respective chloramines (18). Although these substances are more stable in biological systems than O_2^-, H_2O_2, or HCLO, they retain the ability to oxidize sulfhydryl compounds and thioethers and to chlorinate ammonia to yield monochloramine, which is lipophilic and therefore cytotoxic (19).

Exogeneous taurine is an efficient receptor in model systems, and its addition in excess assures that the availability of endogenous amines does not limit the reaction. Chloramines may be quantified by their oxidation of 5-thio-2 nitrobenzoic acid to the disulfide, a change accompanied by a readily-measured shift in absorbance.

Quantitation of taurine chloramine formation appeared to be a suitable method for detection of a postulated inhibition of oxidative activity by toxin, because inhibition could result from an effect on any of the several critical component steps of the overall reaction.

Materials and Methods

Human PMN These were obtained from fresh, heparinized human blood by Ficoll-Hypaque separation, dextran sedimentation, and hypotonic lysis of

erythrocytes, according to the method of Boyum (12).

Opsonized Zymosan Zymosan (Sigma) was suspended at 10 mg per ml in fresh, autologous human serum and held 30 minutes at 37°C with frequent mixing. The mixture was centrifuged, and the sediment was washed twice with Hanks' Balanced salt solution and resuspended at the original concentration.

Determination of Chloramine Formation The method of Weiss et al. (18) was used with minor modifications for study of the effect of anthrax toxin on oxidative activity. The mixtures containing 2.5×10^6 PMN, toxin components in the concentrations indicated, and Dulbecco's phosphate buffered saline to a 1 ml volume were incubated at 37°C in the shaker water broth for the indicated time, after which 50 µl of 2.0 M taurine and 40µl of the opsonized zymosan were added. After incubation for 60 minutes at 37°C, 10µl of 1mg/ml catalase solution was added and the reaction mixtures were centrifuged.

Chloramine in the supernatant was determined by its ability to oxidize 5-thio-2 nitrobenzoic acid (TNB) to 5,5' dithiobis (2 nitrobenzoic acid) (DTNB). TNB was prepared by reduction of DTNB (Sigma Chemical Co.) with cysteine, and determined by measurement of absorption at 412nm (extinction coefficient = $1.36 \times 10^4 M^{-1} cm^{-1}$). The addition of catalase at the end of the reaction period prevented slow oxidation of TNB by residual H_2O_2 (20). Results were expressed in terms of nanomoles of TNB reduced per 10^6 PMN; measurements of effects of toxin on PMN are expressed in relationship to mean values obtained in control mixtures without anthrax toxin. The control values varied somewhat in experiments carried out with different donors on different days.

Results Exploratory experiments revealed that prior incubation of PMN with PA + EF or PA + LF caused inhibition of oxidant formation after the cells were activated by the addition of opsonized zymosan. Experiments were carried out to determine optimum conditions for this reaction. Typical results of

experiments on the time of incubation of PMN + toxin are summarized in Fig. 3, and of concentration of toxin in Fig. 4. At the concentrations studied it is evident that a large part of the inhibitory effect of PA and EF was produced within 30 minutes, whereas the effect of PA + LF was not detectable after 30 minutes, and indeed increased considerably between 60 and 120 minutes. An incubation time of 60 minutes was adequate for the second incubation after addition of zymosan; incubation for shorter periods produced less oxidant, although the degree of inhibition did not change appreciably.

The individual components had no effect on oxidant formation when initial incubation time was short, but increased oxidant formation at 120 minutes. The effects were +17% for PA, +32% for EF; and +49% for LF. These effects have not been studied in detail, and it is not clear whether they represent effects of the toxin components or of contaminants. In any event, the effects are antagonistic to the inhibition observed with PA + EF and PA + LF. Had the stimulative effects of the single component not been present,

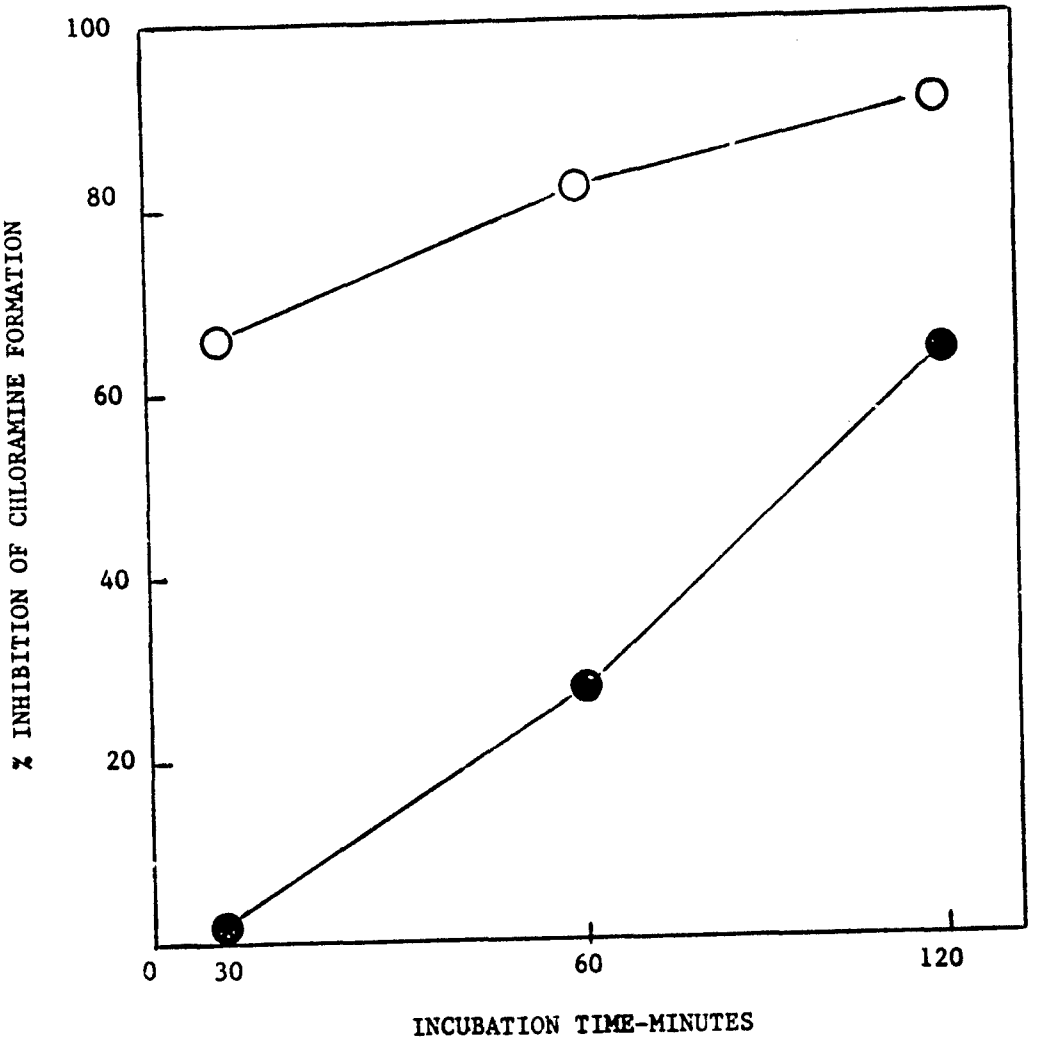


Fig. 3. Effect of time of incubation of neutrophils with toxin on the degree of inhibition of chloramine formation after subsequent stimulation with opsonized zymosan. Incubation was carried out at 37°C with gentle shaking. Results with PA + EF are designated by open circles (mean of 3 experiments). All toxin components were present at 1μg per ml.

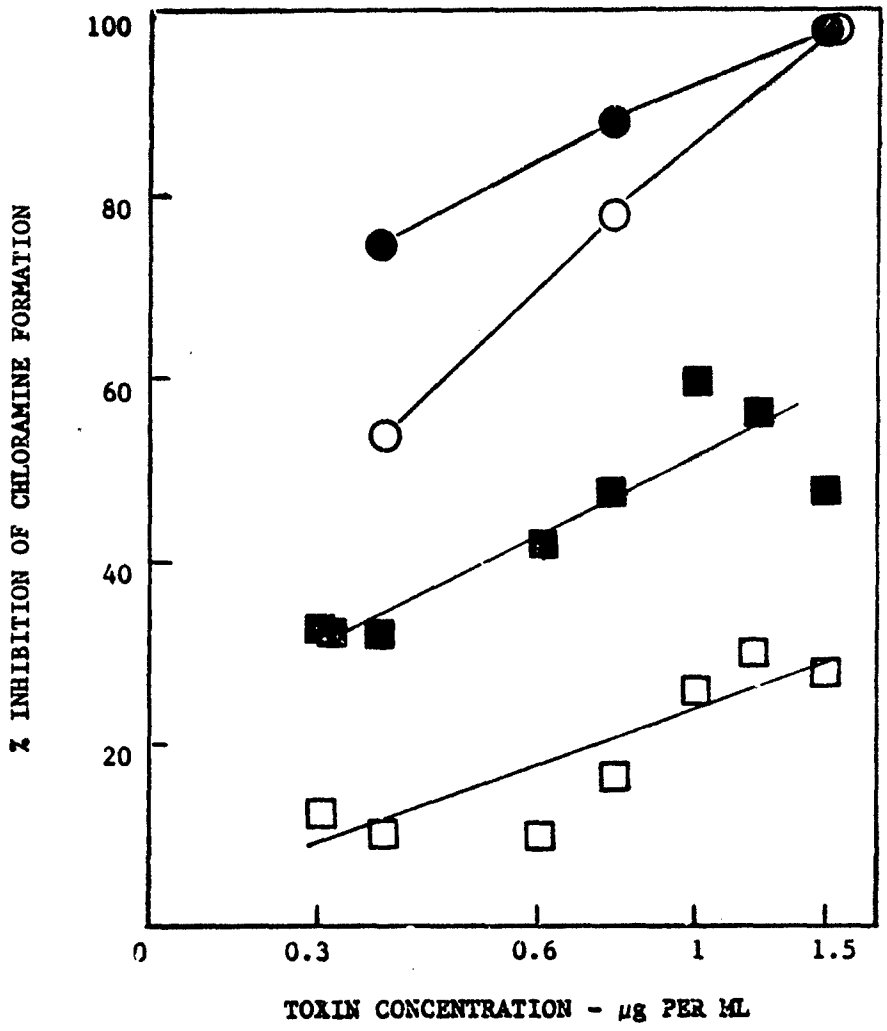


Fig. 4. Effect of concentration of each toxin component on degree of inhibition of chloramine formation by human neuophils after subsequent stimulation with opsonized zymosan. Results with PA + EF are designated by circles, those with PA + LF by squares. Open characters represent 60-minute incubation at 37°C, filled characters 120-minute incubation.

presumably the inhibitory effects of the combinations would have been greater than were observed.

Addition of 0.1mg per ml of superoxide dismutase immediately prior to addition of zymosan increased moderately the formation of oxidant in control mixtures without toxin, but did not alter the percent inhibition produced by toxin treatment. Addition of 0.01 mg per ml of catalase prior to addition of zymosan reduced oxidant formation in control mixture by approximately 40%; the inhibition by toxin was unchanged.

Removal of supernatant fluid from mixtures of toxin and PMN suspension after incubation at 37°C for 30 minutes, followed by resuspension in fresh buffer and reincubation for 90 minutes gave inhibition of oxidant formation equivalent to uninterrrupted incubation for 120 minutes; this provides evidence that inhibition involves a relatively slow reaction after the toxin combines with PMN.

Discussion

The inhibition of chloramine formation observed after treatment of PMN with anthrax toxin provides a possible explanation for the stimulation of chemotaxis reported in Section I of this report. Of even greater interest is the prospect that it may explain the antibactericidal and virulence-enhancing activities of the toxin. The myeloperoxide (MPO) system, on which chloramine formation depends, is regarded as a critical weapon in the armamentarium of the neutrophil (21); an ability to inhibit the MPO system could account for the critical role of anthrax toxin in virulence of Bacillus anthracis.

The MPO system requires halide, hydrogen peroxide, and myeloperoxidase; in theory, the toxin components could act to prevent elaboration, inhibit activity, or stimulate inactivation of any of these components. The observation that addition of an excess of superoxide dismutase did not overcome the inhibition suggests that toxin did not prevent normal functioning

of this enzyme. Efforts are in progress to determine in which component of the overall reaction the effects of toxin are exerted.

Section III. Differential Sensitivity of Target Cells to Adenylate Cyclases of Bacillus anthracis and Bordetella pertussis.

Bordetella pertussis and Bacillus anthracis, while taxonomically unrelated, produce novel adenylate cyclase (AC) toxins which are calmodulin-activated and exert their toxic effects by entering target cells and producing cAMP from endogenous ATP. Although the mechanism of cell entry is as yet unknown, we have noted that all cells are not equally sensitive to intoxication by these toxins. Mammalian cells were exposed to B. pertussis extract containing AC toxin activity, or purified B. anthracis AC, (PA + EF) at maximal concentrations for 1 hour at 37°C.

Intracellular cAMP was extracted and measured by automated RIA.

	Target Cells: cAMP (pmol/mg target cell prot.)			
	CHO	S49	PMN	EL-4
Basal	33 ± 4.8	7.5 ± 0.7	10 ± 1.0	2.4 ± 0.2
B. pertussis				
extract	14,360 + 3,570	12,140 + 870	>10,000	70 + 7.5
EF + PA	11,500 +900	212 +44	52 + 4.0	15.8 + 0.4

Chinese Hamster Ovary (CHO) cells were sensitive to both toxins, while S49 lymphoma cells and human neutrophils (PMN) were markedly more sensitive to B. pertussis AC toxin. EL-4 lymphocyte line was relatively insensitive to both. The effect of these toxins on human monocyte function suggests similar differential sensitivity to that seen with PMN. The differences demonstrated here may reflect cellular differences in surface receptors or intracellular processing of these toxins and will facilitate further study of the uptake mechanism.

ACKNOWLEDGEMENTS

We thank Gail Sullivan for advice and discussion, Agbor Egbewatt and Craig Lombard for assistance, and Lillian Robertson and Margaret Sinex for their contribution to the preparation of this report.

We acknowledge the support and assistance of the University of Virginia Diabetes Center Immuno-assay Core Laboratory for cAMP determinations, and Dr. Donald A. Kaiser for statistical analysis.

REFERENCES

1. Leppla SH. Bacillus anthracis calmodulin-dependent adenylate cyclase: Chemical and enzymatic properties and interactions with eucaryotic cells. In: Greenyard, P. et al, eds. Advances in Cyclic Nucleotide and Protein Phosphorylation Research, New York, Raven Press, Vol. 17: pp 189-198, 1984.

2. Puziss M, Wright GG. Studies on immunity in anthrax X Gel-adsorbed protective antigens for immunization of man. J Bact 85:230-236, 1963.

3. Lincoln RE, Walker JS, Klein F, Rosenwald AJ, and Jones WI Jr. Value of field data for extrapolation in anthrax. Fed Proc 26:1558-1562, 1967.

4. Keppie J, Harris-Smith PW, Smith H. The chemical basis of the virulence of Bacillus anthracis IX: Its aggressins and their mode of action. Brit J Exp Path 44:446-453, 1963.

5. Leppla SH. Anthrax toxin edema factor: A bacterial adenylate cyclase that increases cyclic AMP concentrations in eukaryotic cells. Proc Nat Acad Sci USA 79:3162-3166, 1982.

6. Schiffman E. Leukocyte chemotaxis. Ann Rev Physiol 44:553-568, 1982.

7. Rivkin I, Rosenblatt J, Becker EL. The role of cyclic AMP in the chemotactic responsiveness and spontaneous motility of rabbit peritoneal neutrophils. J Immunol 115:1126-1134, 1975.

8. Bergman MJ, Guerrant RL, Murad F, Richardson DW, Weaver D, Mandell GL. Interaction of polymorphonuclear neutrophils with Escherichia coli. J Clin Inv, 61:227-234, 1978.

9. Confer DL, Eaton JW. Phagocytic impotence caused by an invasive bacterial adenylate cyclase. Science 217:948-950, 1982.

10. Nelson RD, Quie PG, Simmons RL. Chemotaxis under agarose: A new and simple method for measuring chemotaxis and spontaneous migration of human polymorphonuclear leukocytes and monocytes. J Immunol, 115:1650-1656, 1975.

11. Ward PA, Cockrane CG, Muller-Eberhard HJ. The role of serum complement in chemotaxis of leukocytes in vitro. J Exp Med, 122:327-346, 1965.

12. Boyum A. Isolation of mononuclear cells and granulocytes from human blood. Scand J Clin Lab Invest, 21(Suppl. 97):77-89, 1968.

13. Brooker G, Terasaki WL, Price MG. Gammaflow: a completely automated radioimmunoassay system. Science 194:270-276, 1976.

14. Wright GG. Anthrax toxin. In Schlesinger D (ed.), Microbiology-1975. American Society for Microbiology, Washington, D.C., p. 292-295. 1975.

15. Gallin JI, Wright DG, Schiffmann E. Role of secretory events in modulating human neutrophil chemotaxis. J Clin Inv 62:1364-1374, 1978.

16. Clark RA. Extracellular effects of the myeloperoxidase-hydrogen peroxide-halide system. In Wassermann G ed. Advances in Inflammation

Research. New York, Raven Press, Vol. 5:pp. 107-146, 1983.

17. Babior BM. Oxygen-dependent microbial killing by phagocytes. N E J Med 298:659-668; 721-725,1978.

18. Weiss SJ, Lampert MB, Test ST. Long-lived oxidants generated by human neutrophils: characterization and bioactivity. Science 222:625-628 (1983).

19. Grisham MB, Jefferson MM, Melton DF, Thomas EL. Chlorination of endogenous amines by isolated neutrophils. Ammonia-dependent bactericidal, cytotoxic, and cytolytic activities of the chloramines. J Biol Chem 259:10404-10413, 1984.

20. Weiss SJ, Klein R, Slivka A, Wei M. Chlorination of taurine by human neutrophils. J Clin Invest 70:598-607,1982.

21. Babior BM. Oxidants from phagocytes: agents of defense and destruction. Blood 64:959-966, 1984.

DISTRIBUTION LIST

4 copies	Commander US Army Medical Research and Development Command ATTN: SGRD-RMS Fort Detrick, Frederick, Maryland 21701-5012
12 copies	Defense Technical Information Center (DTIC) ATTN: DTIC-DDAC Cameron Station Alexandria, VA 22304-6145
1 copy	Dean School of Medicine Uniformed Services University of the Health Sciences 4301 Jones Bridge Road Bethesda, MD 20814-4799
1 copy	Commandant Academy of Health Sciences, US Army ATTN: AHS-CDM Fort Sam Houston, TX 78234-6100